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October 2, 1956

Dr. John Sharp
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Dear John:

Thanks for your letter. I am glad to see that "proteus 52 recovered" is a problem more than in my own mind. I did not see any bacillary forms in the presence of penicillin, but our conditions are somewhat different. Nor did it give any remarkable yield of L colonies, perhaps for the reasons you mentioned. I did notice that in the absence of penicillin, the extent to which bacillary forms developed depended on the medium (none in plain peptone; about half of the total growth in "penassay" medium) and spent some time thinking about a possible "essential growth factor" for wall formation. But this would be rather elusive. In any case, our coli work is going along rather well now, and I am planning to do nothing more with Proteus: it was very instructive to start.

At the time you were here, I had just gotten some decent primary L colonies from an E. coli strain wg-4, which would have been second-best compared to K-12. Since then, some sublines of K-12 have come through very nicely (giving about 1-10% L colonies per bacterium plated.) So we have gone over completely to these. There is a rather baffling strain variation in the K-12 series, the original K-12 stock being still unproductive: this may have much to do with the mutational basis of L-form competence that you suggested for Proteus. We also had several wild-geese chases with different lots of agar, but it ended up almost trivially: coli needs a stiffer agar than Proteus. It is somewhat startling to see a blank plate with 0.7% agar, and the same full of L-colonies with 0.8% ! (We had been standardised at .75% until now!) Anyhow, we are just starting some experiments today on testing for genetic interactions in L-colony passages of mixtures of well-marked strains.

Some aspects of that first ms. (for PNAS) now leave me rather unhappy, but for various reasons it is too late to alter it. I have submitted the accompanying Note (to J. Bact.) as sort of a postscript, partly to clarify the implicit hypothesis of penicillin action, partly to emphasize the background of work that lies behind it. The Kellenberger article is a first-class job, by the way, though he seems not quite to have grasped the role of osmotic effects (which would have given him 100% yields of protoplasts, and undoubtedly much higher of L colonies). This is surprising, as he quotes a paper by Bonifas which seems to have anticipated both of us on this point. [This seems to be one ~~fixed~~ field which is full of rediscovery: I speak for myself]. Space is at a premium in articles of this kind, but I think the Kellenberger article covers the background rather well.

I hope you are not delaying the publication of your chemical data on the L-forms, and would still like to make a bid to have it for J. Bact. While I realize we may disagree as to their implications for mechanism of action e.g. in of penicillin, I wish it were possible to make an explicit reference to them, this note. The chemical studies have got to be done, of course, on the primary (reversible) protoplasts, but I am sure we will both be surprised if they come out very differently. This still will not define the exact role of penicillin in an enzymatic sense.

The genetic basis of irreversible L-forms is still a puzzle; the only hope I can see of unravelling this is to study them (if they occur) in a bug like E. coli K-12. But I don't believe that the mysterious stabilization of the effect of penicillin is a compelling argument against the proposed action for penicillin itself. It could prove that cell-walls are auto-reproductive, which is other language for your correlation with Ephrussi's yeast story.

Yours sincerely,

Joshua Lederberg